ECVAM/ICCVAM DRAFT PROPOSAL 21 February 2003

THE APPLICATION OF THE PRINCIPLES OF GOOD LABORATORY PRACTICE TO *IN VITRO* TOXICOLOGICAL STUDIES

FOREWORD

In the course of executing its mission to develop, validate and promote *in vitro* methods as alternatives for conventional animal testing, the European Centre for the Validation of Alternative Methods (ECVAM) has taken a leading role in considering quality control issues specifically for *in vitro* studies. *In vitro*, literally, "in glass," refers to a process that takes place under artificial conditions often outside of the living organism. In 1999, ECVAM held a workshop on "The Principles of Good Laboratory Practices: Application to *In Vitro* Toxicology Studies". The report of this workshop was forwarded to OECD and served as the basis for this document. The intent of this proposal is to illustrate that clear additional guidance on Good Laboratory Practice (GLP) is needed for the exponentially growing area of *in vitro* studies that would be conducted for regulatory purposes world-wide. It also cites the appropriate OECD Principles of GLP and gives guidance on their interpretation in relation to *in vitro* toxicological studies. The U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), through its GLP Subcommittee, concurs with this concept and strongly supports this proposed project. Therefore, both ECVAM and ICCVAM present via this proposal their joint ECVAM/ICCVAM position. Although in the GLP Consensus Document, "The Application of the GLP Principles to Short-Term Studies," some attention is paid to *in vitro* methods, this proposal indicates why a separate GLP Consensus Document, 'The Application of GLP Principles to *In Vitro* Toxicological Studies' would be of considerable value.

An OECD Consultation meeting between the Members of the OECD Working Group on GLP and ECVAM/ICCVAM on the need for further guidance on the application of the Principles of GLP on *in vitro* studies will take place on the 4th of March, OECD, Paris. Members of the consultation group for ECVAM are Sandra Coecke and Thomas Hartung and for ICCVAM, Leonard Schechtman and William Stokes.

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BACKGROUND

The OECD Principles of GLP are general and not specific to any particular type of testing discipline. The experience in OECD Member countries in compliance monitoring has been primarily in animal studies. During the last decade, there has been increasing focus throughout the international scientific and regulatory communities on the development of *in vitro* toxicological test methods for regulatory decision making. Although subject to the OECD Principles of GLP, these *in vitro* methods, place special emphasis on the test system and milieu (i.e., the cells, tissues, or organs in culture), with regard to verification of identity, absence of contamination or defects, replicates, performance, reproducibility over time and across different types of test substances. Furthermore, quality control of the test system is an essential element in any study conducted for regulatory purposes to ensure that the results of the study are meaningful and comparable to data from previous studies and/or across laboratories. Procedures for formal, systematic validation of these new alternative methods have been developed by ECVAM in Europe and ICCVAM in the United States. In addition, OECD has published practical guidance on validation for use internationally (OECD GD No.34).

Experts involved in the conduct of *in vitro* studies (ECVAM Workshop Report 37, "The Principles of Good Laboratory Practice: Application to *In Vitro* Toxicology Studies," 1999) have suggested that additional guidance is needed to cover the application of GLPs to specific aspects of *in vitro* studies. Additional relevant considerations are provided in an ECVAM Task Force Report on Good Cell Culture Practices (Hartung *et al*., 2002).

The OECD "Consensus Document on the Application of the GLP Principles to Short Term Studies" is directed at short-term studies (i.e., studies of short duration that employ widely used, routine techniques) for both *in vivo* and *in vitro* test methods. However, *in vitro* studies are not necessarily limited to studies of short duration or to those that would be based on widely used, routine techniques. For example, some *in vitro* neoplastic transformation assays take between one and two months to conduct. It is also conceivable that *in vitro* systems or *in vitro* test batteries will be developed targeting long-term effects such as the *in vitro* assessment of systemic toxicity over an extended timeframe, assessment of repeated-dose effects, and assessment of post-treatment recovery.

The acceptance in 2002 of four new OECD test guidelines for *in vitro* methods by the OECD WNT National Coordinators for the Test Guidelines Program points to the need for a stand-alone OECD document on "The Application of the Principles of Good Laboratory Practice to *In Vitro* Toxicological Studies". In the last few years, there has been a considerable increase in the development of *in vitro* assays (both proprietary and non-proprietary), and this can be expected to accelerate as new technologies, high throughput methods, and other alternative methodologies are incorporated into testing approaches that lend themselves to research and regulatory purposes (e.g., toxicogenomics, proteomics, nano-technologies, etc.).

Users and auditors of these *in vitro* test methods for regulatory purposes, need to be assured that these methods perform well over time and provide consistent high quality data. Since some of these methods contain proprietary materials or are available as proprietary test kits, the manufacturer or supplier of these must have a good quality control programme in place. However, the collection and presentation of the quality control information is the responsibility of the testing facility submitting data in fulfilling regulatory requirements.

Some of the available cell, tissue, and organ models have a limited life-time and are relatively expensive, which leads to practical limitations in the number of replicates used for quality control. Often the user depends on the manufacturer or supplier for cell, tissue, or organ characterisation and quality control. Therefore, for regulatory purposes, it is essential that negative and positive control substances be used for monitoring the performance of the test insuring its validity. In order to compare the study results for different test substances, the performance of the controls should be consistent over time or from preparation to preparation of the cells, tissues, and organs.

NOTES TO THE GLP PRINCIPLES

Examples of further guidance and interpretation applicable to in vitro toxicological studies are provided below as NOTES for specific paragraphs of the OECD Principles of GLP. Paragraphs of the OECD Principles which may not require further guidance and interpretation are not repeated here

1. TEST FACILITY ORGANISATION AND PERSONNEL

1.2. Study Director's responsibilities

1.2.2.f) (The Study Director should) ensure that all raw data generated are fully documented and recorded.

[NOTE]: For *in vitro* studies using proprietary materials or test kits, Study Directors should ensure proper functioning of the test method. Appropriate reference items, including negative and positive controls, should be used for this purpose. The completeness and acceptability of delivered quality control documents provided by suppliers of proprietary methods and test kits should be evaluated.

2. QUALITY ASSURANCE PROGRAMME

2.1. General

2.1.1. The test facility should have a documented Quality Assurance Programme to assure that studies performed are in compliance with these principles of Good Laboratory Practices.

[NOTE]: The documentation of the quality assurance programme should include in the "study-based", "facility-based" and "process-based" issues specifically relevant to *in vitro* toxicological studies.

***Study-based inspections*: Detailed *in vitro* test system characterisation is a crucial aspect related to *in vitro* toxicological studies. It is necessary to ensure that the quality of all components involved, including the cells or tissues, the equipment and cell and tissue culture materials, are sufficient to assure accurate and reproducible results. Slight variations in equipment and cell and tissue culture materials can impact the study results, and therefore should be documented and monitored. Specific examples include:**

- **changes of batches of cell and tissue culture materials, test systems and other supplies should be monitored with regard to their influence on in vitro growth conditions and principal endpoints in the study;**
- **measures should be taken to assess the integrity and status of cells and tissues before any experiment is undertaken;**
- **solvent and positive control items should be included in all experiments, and benchmark materials and negative controls where appropriate;**
- **time in culture of tissues, cell seeding density, subcultivation intervals and passage number should be defined;**

- **cell and tissue culture medium volumes and feeding cycles should be defined and documented; and**
- **if applicable, specific cell and tissue culture surface requirements should be documented and monitored for specific quality aspects.**

***Facility-based inspections*: *In vitro* studies do not normally require dedicated facilities that are exclusionary of other studies, and equipment and supplies are often shared among in vitro studies:**

- **equipment and environmental control monitoring measurements need to be continuous or frequent, rather than sporadic ; and**
- **a monitoring programme should be in place for the detection of mycoplasma contamination, and if applicable, other contaminants. Special concerns exist for continuous cell lines to carry latent viruses, and for transformed cell lines to spontaneously produce viruses with oncogenic potential in humans. Human-derived cells and tissues should be appropriately monitoring for biohazards (e.g., HIV, Hepatitis B) Monitoring methods, testing intervals, and control and safety procedures should be documented.**

***Process-based inspections*: Processes of a repetitive nature in *in vitro* studies include:**

- **proper use of laminar-flow cabinets;**
- **proper handling of liquid nitrogen during cryopreservation of cells and tissues and retrieval of vials from frozen storage;**
- **proper handling of cell and tissue derived waste; and**
- **proper measures and indicators to ensure the sterility of non-disposable cell and tissue culture materials and supplies.**

3. FACILITIES

3.1. General

3.1.1. The test facility should be of a suitable size, construction and location to meet the requirements of the study and to minimise disturbance that would interfere with the validity of the study.

3.1.2. The design of the test facility should provide an adequate degree of separation of the different activities to assure the proper conduct of each study.

[NOTE]: Due to the fact that *in vitro* studies do not normally require dedicated facilities that are exclusionary of other studies, measures should be taken to ensure the appropriate separation of co-existing *in vitro* studies in the same physical environment.

3.2. Test System Facilities

3.2.1. The test facility should have a sufficient number of rooms, areas to assure the isolation of the test system and the isolation of the individual projects, involving substances or organisms known to be or suspected of being, biohazardous.

[NOTE 1]: The test system for *in vitro* toxicological studies is often maintained within an incubator, which provides the required environmental conditions.

[NOTE 2]: The test systems used in different studies can often be maintained in the same incubator, provided there is adequate isolation of test systems cultures by the flasks, tubes or plates in which the cells and tissues are grown or treated.

3.2.2. Suitable rooms, areas should be available for the treatment of the test systems in order to ensure that there is no contamination of the test system (s).

[NOTE]: Most *in vitro* cell and tissue culture systems used for toxicological studies are manipulated in vertical biohazard laminar air flow cabinets to assure sterility and protection of the test system, study personnel and environment. Test systems used for different projects should not be handled concurrently in the same air flow cabinet.

3.3. Facilities for Handling Test and Reference Items

3.3.1. To prevent contamination or mix-ups, there should be separate rooms or areas for receipt and storage of the test and reference items.

[NOTE]: To assure sterility of the test system during studies executed over longer periods, test and reference items will be handled or manipulated via special measures. Mixing of the test and reference items with a vehicle should be performed so as to preclude contamination and mix-up.

4. APPARATUS, MATERIALS AND REAGENTS

4.2. Apparatus used in a study should be periodically inspected, cleaned, maintained, and calibrated according to Standard Operating Procedures. Records of these activities should be maintained. Calibration should, where appropriate, be traceable to national or international standards of measurement.

4.3. Apparatus and materials used in a study should not interfere adversely with the test systems.

[NOTE]: The apparatus used to maintain test system isolation and environment and the cell and tissue culture reagents and materials are particular critical for *in vitro* studies. Issues include:

- **cell and tissue culture equipment and instruments should be maintained and calibrated properly (e.g., control of temperature and CO_2 levels of incubators);**
- **all cell and tissue culture materials employed should be stored under appropriate conditions to protect them from damage, infestation, or contamination;**
- **composition of culture media for routine cultures (maintenance media) and/or experimental cultures, and supplements/additives (e.g., serum, heat-inactivation or irradiation of serum, growth factors, hormones, antibiotics) should be defined, to the extent possible;**
- **names and addresses of manufacturers and suppliers of culture media, media supplements, culture substrata and culture vessels should be documented.**

5. TEST SYSTEMS

5.2. Biological

5.2.1. Proper conditions should be established and maintained for the storage, housing, handling and care of the biological test systems, in order to ensure the quality of the data.

[NOTE]: Where appropriate, culture vessels (flasks, Petri dishes, bottles, roller cultures, etc.) and storage vials or containers should be defined and sterility methods should be documented and verified. Monitoring systems should be established to assure the adequate storage conditions of cell and tissues used for *in vitro* studies (e.g., refrigerators, freezers, cryopreservators, etc…).

5.2.2. Newly received animal and plant test systems should be isolated until their health status has been evaluated. If any unusual mortality or morbidity occurs, this lot should not be used in studies and, when appropriate, should be humanely destroyed. At the experimental starting date of the study, test systems should be free of any disease or condition that may interfere with the purpose or conduct of the study. Test systems that become diseased or injured during the course of a study should be isolated and treated, if necessary to maintain the integrity of the study. Any diagnosis and treatment of any disease before or during the study should be recorded.

[NOTE 1]: Newly received *in vitro* cells and tissues should be controlled and monitored for their purity, lack of contamination, suitability and identity. Until the *in vitro* test system has met these pre-defined criteria, it should not be used in GLP-compliant studies. At the test facility, information needs to be available on monitoring methods and intervals for screening for mycoplasma and other adventitious agents, and other appropriate monitoring methods to ensure the use of non-contaminated cells and tissues. When applicable, verification is necessary on the types of cells or tissues used in *in vitro* toxicological studies and their specific properties that make them appropriate for the study for which they are intended. For this purpose, morphological evaluation, control for the stability of the phenotype, absence of infections during study, control for dedifferentiation, and expected population doubling times may be used.

[NOTE 2]: For *in vitro* studies based on test systems that contain proprietary materials or proprietary test kits, it is essential that the receiving test facility verifies the quality of the *in vitro* test system upon receipt by using appropriate quality control measures.

5.2.3. Records of source, date of arrival, and arrival conditions of test systems should be maintained.

[NOTE]: Many *in vitro* test systems are not directly isolated by the test facility itself, but are cells or tissues obtained from other sources. In these cases, the cell or tissue repository or commercial source might be the primary source of data on the species and tissue of

origin. It is essential that the test facility is able to trace the *in vitro* test system to its immediate source. At the test facility information needs to available on:

- **origin or source of the cell or tissue (e.g., reconstituted tissue from a commercial source, primary tissue explant; established cell line from a cell bank such as ATCC, ECACC, DMSZ, Riken Gene bank; depositor; laboratory of origin; original publication/patent);**
- **nomenclature of the cell or tissue in use and its related lot number (e.g., ATCC designation, designation given in-house or by commercial source);**
- **method by which cell or tissues were obtained (i.e. cells derived from a tissue biopsy or explant, shipped frozen or in liquid medium, reconstituted tissue, cell lines derived from carcinoma);**
- **chronology of custody (historical tracking) of cell or tissue culture, where applicable; and**
- **mode of culture initiation (species, organ, tissue, lineage, mode of transformation, genetic modification, sublines/hybrid cells; in case of humans: donor characteristics such as race, sex, age, disease, health status and medication, biopsy, tumour, if such information is available);**

5.2.4. Biological test systems should be acclimatised to the test environment for an adequate period before the first administration/application of the test or reference item.

[NOTE]: This issue is of specific concern when cells or tissues are received from the cell or tissue repository or commercial source in a frozen form. Conditions for freezing/thawing, including cryoprotectant, storage conditions, viability, plating/cloning efficiency (if available) must be documented. Measures have to be taken to assure the adequate propagation of the *in vitro* test system after receipt and should be consistent with the study plan and/or Standard Operating Procedure.

5.2.5. All information needed to properly identify the test systems should appear on their housing or containers. Individual test systems that are to be removed from their housing or containers during the conduct of the study should bear appropriate identification.

[NOTE]: Culture vessels (flasks, Petri dishes, bottles, roller cultures, etc.) and storage vials or containers should be properly labelled to properly identify the *in vitro* test system. In cases of storage in refrigerators, freezers, liquid nitrogen measures should be taken to avoid any detachment of labels. Especially in the case of storage of the *in vitro* test systems in cryovials with a limited dimension in liquid nitrogen, measures should be undertaken to assure adequate labelling and prevention of detachment of labels. Proper handling of liquid nitrogen during cryopreservation of cells and tissues and retrieval of vials or containers from frozen storage is essential.

5.2.6. During use, housing or containers for test systems should be cleaned and sanitised at appropriate intervals. Any material that comes into contact with the test system should be free of contaminants at levels that would interfere with the study. Bedding for animals should be changed as required by sound husbandry practice. Use of pest control agents should be documented.

[NOTE]: Proper conditions should be established and maintained for the handling of the *in vitro* test system. Good cell and tissue culture practices and good aseptic techniques, which are an essential part of *in vitro* work must be enforced and monitored. Specific attention has to be paid with regard to documentation of:

- **culture conditions and subcultivation intervals (volume of medium on cell and tissue cultures, cell density confluent/subconfluent, cell cultures, cell harvest, split ratio, initial passage number, number of passages in culture, time of tissue in culture, effect of passage number on principal endpoint of test);**
- **indicators of the state of differentiation and expressed activities, where available;**
- **precise definition of measures undertaken to maintain or induce differentiation.**

6. TEST AND REFERENCE SUBSTANCES

6.2. Characterisation

6.2.1. Each test and reference item should be appropriately identified (e.g., code, Chemical Abstracts Service Registry Number [CAS number], name, biological parameters).

[NOTE 1]: For *in vitro* studies the term reference items includes benchmark materials, and solvent, negative, and positive control items. Control items serve in monitoring the performance of the *in vitro* test system(s), but might not necessarily be compared with the test item(s) in the same way as the conventional reference item. In many situations, only the positive control items are classified as separate item(s), since the solvent control items might just be diluent/medium used to prepare the test item(s), the positive and negative controls, and/or the reference items.

[NOTE 2]: For *in vitro* studies in general, but especially for tests that contain proprietary materials or proprietary test kits, the use of positive and solvent control items are usually essential, and negative controls may also be necessary. The positive control item should be chosen in such a way that it can detect any over- or under-responses in the test system predetermined from historical values and related to the required precision of the test. Additional reference items (e.g., bench mark materials) can be used to set upper or lower limits of acceptable responses against which the unknown test item may be judged. In some test systems, it may be appropriate to specify the use of reference items that are of a similar class to that of the unknown test item. This can serve as an additional performance control of the test system for that class of material.

7. STANDARD OPERATING PROCEDURES

[NOTE]: Beside study-based procedures, several process-based Standard Operating Procedures need to be established. Examples include Standard Operating Procedures for sterile/aseptic working conditions, use of laminar-flow cabinets and cell and tissue incubators, screening for mycoplasma and other adventitious agents, handling of liquid nitrogen, storage of cell and tissues etc…

8. PERFORMANCE OF THE STUDY

[NOTE]: The response of an *in vitro* test system to reference items (e.g., benchmark materials, solvent, negative and positive control items) provides the basis for determining the acceptability of an assay. The observed positive control response is compared to historical values to determine whether or not the response falls within the predefined limits given in the study plan's acceptance criteria.

9. REPORTING OF STUDY RESULTS

[NOTE]: Reports of *in vitro* toxicological studies should be written taken into account all principles related to good cell and tissue culture practices.
Specific attention has to be paid with regard to reporting of:

***In vitro* test system (cells and tissues)**

- **origin or source of the cell or tissue (e.g., reconstituted tissue from a commercial source, primary tissue explant; established cell line from a cell bank such as ATCC, ECACC, DMSZ, Riken Gene bank; depositor; laboratory of origin; original publication/patent);**
- **nomenclature of the cell or tissue in use and its related lot number (e.g., ATCC designation, designation given in-house or by commercial source);**
- **method by which cell or tissues were obtained (i.e. cells derived from a tissue biopsy or explant, shipped frozen or in liquid medium, reconstituted tissue, cell lines derived from carcinoma);**
- **chronology of custody (historical tracking) of cell or tissue culture, where applicable; and**
- **mode of culture initiation (species, organ, tissue, lineage, mode of transformation, genetic modification, sublines/hybrid cells; in case of humans: donor characteristics such as race, sex, age, disease, health status and medication, biopsy, tumour, if such information is available);**
- **basic morphological description of cultured cells, including stability of the phenotype and expected population doubling times;**
- **culture conditions and subcultivation intervals (cell density, confluent/subconfluent cultures, cell harvest, split ratio, initial passage number, number of passages in culture, effect of passage number on principal endpoint of test);**
- **culture conditions and subcultivation intervals (volume of medium on cell and tissue cultures, cell density confluent/subconfluent, cell cultures, cell harvest, split ratio, initial passage number, number of passages in culture, time of tissue in culture, effect of passage number on principal endpoint of test);**
- **indicators of the state of differentiation and expressed activities, where available;**
- **precise definition of measures undertaken to maintain or induce differentiation; and**
- **test methods and test interval for screening for mycoplasma and other adventitious agents and other appropriate tests to ensure the use of non-contaminated cell and tissues.**

Other materials, equipment and procedures

- **slight variations of these materials can impact study results, and therefore should be monitored and documented;**
- **equipment and instruments should be maintained and calibrated properly (e.g., control of temperature and CO2 levels of incubators);**
- **all materials employed should be stored under appropriate conditions to protect them from damage, infestation, or contamination;**
- **composition of culture media for routine cultures (maintenance media) and/or experimental cultures, and supplements/additives (e.g., serum, heat-inactivation or irradiation of serum, growth factors, hormones, antibiotics) should be defined, to the extent possible;**
- **non-defined preparations (e.g., serum-replacements, growth factor mixtures) should not be considered acceptable;**
- **cell and tissue culture medium volumes used and feeding cycles must be defined;**
- **changes of batches of materials must be monitored with regard to their influence on *in vitro* growth conditions, inhibitory factors, and principal endpoints of the study;**
- **culture vessels (flasks, Petri dishes, bottles, roller cultures, etc.) must be defined and sterility methods should be documented and verified;**
- **culture substrata and/or coating materials/procedures (e.g., collagen, fibronectin, laminin) must be defined;**
- **names and addresses of manufacturers and suppliers of culture media, media supplements, culture substrata and culture vessels must be documented; and**
- **lot-to-lot consistency of materials and supplies should be documented and verified.**